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(54) Title: METHOD OF SENSITIZING TUMOR CELLS WITH ADENOVIRUS E1A

(57) Abstract

The present invention is directed to methods of sensitizing a human tumor cell with adenovirus E1A. The methods involve treating a human tumor cell by, first, introducing into the tumor cell nucleic acid encoding a polypeptide having adenovirus E1A activity, expressing the E1A active polypeptide in the cell, and then either contacting the E1A expressing tumor cell with a chemotherapeutic agent or irradiating the E1A-expressing tumor cell. The invention also provides methods of enhancing a subject's response to chemotherapy or irradiation by introducing into a subject's tumor cells nucleic acid encoding a polypeptide having adenovirus E1A activity, expressing the E1A active polypeptide in the cells and finally, administering either a chemotherapeutic agent or irradiation. The invention also provides a method of treating cancer.

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METHOD OF SENSITIZING TUMOR CELLS WITH ADENOVIRUS E1A

The present invention was made with support in part from a National Institutes of Health Grant R29GM44573-05. The United States Government may have certain rights in the invention.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the fields of molecular biology and more specifically to the effect of adenovirus E1A on tumor cells.

BACKGROUND INFORMATION

Both radiation and chemotherapy have significantly contributed to the treatment of cancer. However, impediments to successful therapy by either form of treatment still remain. For example, some tumor types fail to respond to either radiation or chemotherapy. In other instances, originally responsive malignant cells may experience a relapse and become resistant to treatment.

There is evidence that the expression of certain proteins, such as various oncogenes, can increase the susceptibility of cells to apoptosis, also known as programmed cell death. Further, there is evidence that some of these proteins may also confer susceptibility to apoptosis induced by anticancer agents. However, relatively few studies have been done to observe these

effects on human tumor cells and there have been no reports on the effect of adenovirus E1A on the sensitivity of human tumor cells and the response of these E1A-expressing cells to chemotherapeutic agents or irradiation.

Given the shortcomings of current chemotherapy and irradiation, namely lack of response and resistance or tolerance to chemotherapy agents, there is a need for developing additional forms of treatment which can enhance a response to chemotherapy or irradiation.

The present invention satisfies these needs and provides related advantages as well. The present invention provides novel methods for treating a tumor cell and enhancing a patient's response to irradiation and chemotherapy.

SUMMARY OF THE INVENTION

The present invention is directed to methods of sensitizing a human tumor cell with adenovirus E1A. The methods involve treating a human tumor cell by, first, introducing into the tumor cell nucleic acid encoding a polypeptide having adenovirus E1A activity, expressing the E1A active polypeptide in the cell, and then either contacting the E1A expressing tumor cell with a chemotherapeutic agent or irradiating the E1A-expressing tumor cell.

The invention also provides methods of enhancing a subject's response to chemotherapy or irradiation by introducing into a subject's tumor cells nucleic acid encoding a polypeptide having adenovirus E1A activity, expressing the E1A active polypeptide in the cells and finally, administering either a chemotherapeutic agent or irradiation. This invention also provides a method of treating cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides etoposide cytotoxicity dose-response curves of various tumor cell lines or E1A-expressing derivatives, including HT 1080 fibrosarcoma (FS), A2058 melanoma (ML), SaoS-2 osteosarcoma (OS), and NCI-H23 non-small-cell lung carcinoma (LC).

Figure 2 provides additional evidence of the effect of E1A-expressing human A2058 melanoma cells to etoposide. Comparisons were made by agarose gel electrophoresis of DNA extracted from A2058 melanoma cells in the absence (-) or presence (+) of E1A and the absence (-) or presence (+) of etoposide.

Figure 3 provides evidence of stable transfection and expression of E1A in various tumor cells by immunoprecipitation of E1A proteins from stably transfected and parental cell lines. The bands corresponding to E1A protein species are bracketed.

Figure 4 depicts the structure of an E1A reverse-transforming retrovirus construct.

Figure 5 provides the DNA sequence of E1A coding sequences (cDNA). Bottom line: 243 amino acid sequence. Top line: 289 amino acid sequence.

Figure 6 provides evidence of E1A protein expression in HT1080 fibrosarcoma (FS), E1A+ derivative cells as compared to the parental HT1080 cell line (E1A-).

DETAILED DESCRIPTION OF THE INVENTION

Adenovirus is a large DNA virus whose natural host is human cells. Virtually every adult has been infected with adenovirus at some time, the major effect being cold-like symptoms. Adenovirus is referred to as a "DNA tumor virus" because of its oncogenic effect in rodents. Expression of the adenovirus genome occurs in two stages. First, the early gene products are expressed which encode the E1A and the E1B genes. These products are necessary for expression of the late gene products. Late gene products encode proteins necessary for replication as well as the viral structural proteins.

The proteins encoded by the E1A gene of adenovirus have been studied primarily from two points of view. First, the 243 amino acid and 289 amino acid forms of E1A (arising from alternative splicing of the precursor RNA such that the 243 amino acid protein is a subset of the 289 amino acid protein) are both transcriptional regulatory

proteins, J. Flint, T. Shenk, Ann. Rev. Gen., 23:141-161 (1989). Secondly, these proteins facilitate the oncogenic transformation of certain rodent cells by other oncogenes, H.E. Ruley, Nature, 304:602-606 (1983), and, as such E1A is generally classified as an oncogene.

There is evidence that the expression of oncogenes can increase the susceptibility of cells to apoptosis, also known as programmed cell death. Lowe et al., Cell, 74:957-967 (1993). For example, the E1A gene may increase cellular susceptibility to apoptosis in primary rodent cells. Roa et al., Proc. Natl. Acad. Sci., 89:7742-7746 (1992). Other oncogenes, such as *c-myc*, can also increase cellular susceptibility to programmed cell death, Evan et al., Cell, 69:119-128 (1992), and overexpression of *c-myc* may also confer susceptibility to apoptosis induced by anticancer agents, such as tumor necrosis factor α, Chen et al., Nature, 330:581-583 (1987), or etoposide, Fanidi et al., Nature, 359:554-556 (1992) and Lowe et al., *supra*. Additionally, Lowe et al., *supra*, explore the effects of p53 in combination with E1A on the sensitivity of mouse embryo fibroblasts (MEFs) to chemotherapeutic drugs.

Interestingly, and in contrast to E1A's oncogenic and apoptotic effects in rodent cells, E1A acts as a tumor suppressor gene in the human context. Steven M. Frisch, Proc. Natl. Acad. Sci., 88:9077-9081 (1991), which is incorporated herein by reference, provides evidence of the antioncogenic effect of adenovirus E1A in human tumor cells. More importantly, it is striking and unexpected

that E1A, sensitizes human tumor cells and enhances tumor cell's response to chemotherapy and irradiation treatment. The effect is independent of p53.

These unexpected observations provide the basis for this invention, which is directed to methods of sensitizing a human tumor cell with adenovirus E1A. The methods involve treating a human tumor cell by, first, introducing into the tumor cell nucleic acid encoding a polypeptide having adenovirus E1A activity, expressing the E1A active polypeptide in the cell, and then either contacting the E1A-expressing tumor cell with a chemotherapeutic agent or irradiating the E1A-expressing tumor cell. The methods may be practiced in vitro or in vivo.

Also provided by this invention are methods of enhancing a subject's response to chemotherapy or irradiation by introducing into a subject's tumor cells nucleic acid encoding a polypeptide having adenovirus E1A activity, expressing the E1A active polypeptide in the cells and finally, administering either a chemotherapeutic agent or irradiation.

The invention also provides methods of treating a cancer which is susceptible to treatment by introducing into a subject's tumor cells nucleic acid encoding a polypeptide having adenovirus E1A activity, expressing said polypeptide in said tumor cells, and finally, either contacting said tumor cells with a chemotherapeutic agent or irradiating said tumor cells. Thus, a nucleic acid

molecule encoding a polypeptide having E1A activity can be useful in the preparation of a medicament for enhancing a subjects response to chemotherapy or irradiation and for the treatment of cancer.

Treating human tumor cells with E1A sensitizes the cells. The sensitization results in enhancing a tumor cell's susceptibility to respond to chemotherapeutic agents or irradiation. As evidenced in the ensuing Examples, by the instant methods E1A-expressing cells more readily sensitize human tumor cells and make them more susceptible to killing by, chemotherapy or irradiation as compared to cells lacking E1A. Of course, it will be understood that chemotherapeutic agents or irradiation may affect tumor cells by a variety of ways, including killing or decreased viability, by for example apoptosis or other mechanisms, poisoning, inactivation of tumor, and the like. It is expected, regardless of the mechanisms by which the toxic agents (chemotherapeutic agents or irradiation) work, E1A-expression sensitizes the cells and enhances their response to chemotherapy or irradiation. While not wishing to be bound by any theory or mechanism, it is postulated that the instant invention may work based on E1A's ability to reverse-transform human tumor cells and convert them to epithelial cells. Reverse-transformation by E1A confers sensitivity to anoikis (a type of programmed cell death). Frisch and Francis, J. Cell Biology, 124:619-626 (Feb. 1994).

One can easily test for sensitization by assays well known and readily available in the art, such as for example by a dose response assay to assess cell viability or agarose gel electrophoresis of DNA extractions to determine DNA fragmentation, a characteristic of cell death. Both of these assays are further described in the Examples below. Other assays, such as a chromatin assay, which is well known in the art, or drug resistance assays (as described, for example, in Lowe et al., Cell, 74:957-967 (1993), which is incorporated herein by reference) may also be used to determine the effect of E1A on the sensitivity of tumor cells and their response to E1A and chemotherapy agents or irradiation.

As used herein, the term "nucleic acid" means DNA, including cDNA, or RNA. Isolated nucleic acids useful in this invention are those that encode a polypeptide functionally equivalent to a polypeptide encoded by the E1A region of the adenovirus genome. In one aspect of this invention, the isolated nucleic acid is the adenovirus E1A region. This region is defined by those of skill in the art to be from nucleotide 560 to nucleotide 1542. The nucleotide and amino acid sequence of E1A is provided in Figure 5.

As used herein, the term "introducing" in relation to nucleic acid encompasses any method of inserting an exogenous nucleic acid molecule into a cell and includes, but is not limited to transduction, transfection, microinjection and viral infection of host cells. Methods of carrying out these procedures are well

known to those of skill in the art. The nucleic acid may be introduced, for example, by contacting the cell with an adenovirus vector or a retroviral vector. In a preferred embodiment, the nucleic acid is introduced into the cell by contacting a cell with a retroviral vector containing the nucleic acid under conditions such that the nucleic acid is inserted into the cell. In the most preferred embodiment of this invention, the virus is a replication-incompetent retrovirus. A replication-incompetent retrovirus is defined as a virus not having the ability to produce viral proteins, precluding spread of the vector in the infected host cells. Examples of such vectors useful for the practice of this invention are well known and readily available to those of skill in the art.

The E1A gene product has the capacity, after being expressed in a human tumor cell to sensitize the tumor cell and enhance the cell's response to a chemotherapeutic agent or irradiation. Such capacity is herein termed "E1A activity." A "polypeptide having E1A activity" is used in its broadest sense to include the structure of native adenovirus E1A, its functional subunits and fragments and modifications to the native sequence which retain E1A activity. In one aspect of the invention, this amino acid sequence is the 243 amino acid polypeptide product of the adenovirus E1A gene, as provided in Figure 5. Functional equivalents of the 243 amino acid polypeptide are polypeptides that can also sensitize human tumor cells and enhance their susceptibility to treatment by chemotherapy agents or irradiation. Cells which express a polypeptide having E1A activity are repeatedly referred

to herein as "E1A-expressing" cells. The activity of an E1A polypeptide can be routinely determined by any one of the tests for sensitivity and/or response to cancer treatment as described above and below.

The invention relates generally to a method of sensitizing a human tumor cell based on the effect of E1A and the response of E1A-expressing cells to a toxic agent, i.e., chemotherapeutic agent or irradiation. As used herein, the phrase "chemotherapeutic agent" means any chemical agent or drug used in chemotherapy treatment which selectively affects tumor cells, including but not limited to such agents as adriamycin, amsacrine, etoposide, actinomycin D, VP 16, camptothecin, colchicine, taxol, cisplatinum, vincristine, vinblastine, methotrexate. Other such agents are well known in the art. As described above, the agents encompassed by this invention are not limited to working by any one mechanism, and may for example be effective by direct poisoning, apoptosis or other mechanisms of cell death or killing, tumor inactivation, or other mechanisms known or unknown. The means for contacting tumor cells with these agents and for administering a chemotherapeutic agent to a subject are well known and readily available to those of skill in the art.

As used herein, the term "irradiation" or "irradiating" is intended in its broadest sense to include any treatment of a tumor cell or subject by photons, electrons, neutrons or other ionizing radiations. These radiations include, but are not limited to, X-rays, gamma-

radiation, or heavy ion particles, such as alpha or beta particles. Moreover, the irradiation may be radioactive, as is commonly used in cancer treatment and can include interstitial irradiation. The means for irradiating tumor cells and a subject are well known and readily available to those of skill in the art.

The methods described herein can be useful for sensitizing cancers of a number of types, including but not limited to breast cancer, sarcomas and other neoplasms, bladder cancer, colon cancer, lung cancer, various leukemias and lymphomas. The tumor cells of the instant invention are from human tumors or malignancies. In one aspect of this invention, human tumor cells do not express functional p53. Human tumor cells which do not express functional p53, include, but are not limited to, HT1080 fibrosarcoma, Saos2 osteosarcoma, NCI-H23 non-small cell lung carcinoma, and RD rhabdomyosarcoma. Evidence that these cell types do not express functional p53 can be found as follows: HT1080: Anderson et al., Genes, Chromosomes & Cancer, 9:266-281 (1994), which is incorporated herein by reference; Saos-2: Subler and Martin, J. of Virology, 68:103-110 (1994), which is incorporated herein by reference; RD: Felix et al., Cancer Research, 52:2243-2247 (1992), which is incorporated herein by reference; and NCI-H23: Takahashi et al., Cancer Research, 52:2340-2343 (1992), which is incorporated herein by reference. One would expect that any other human tumor cell and particularly ones which do not express functional p53 would be sensitized by E1A and would enhance the cells' response to chemotherapeutic drugs or irradiation.

The phrase "functional p53" means any structure which substantially corresponds to the native p53 protein and which has the function of native p53, which is generally known to be tumor suppression. Therefore, mutations in p53 which result in a lack of p53 activity or function would not be encompassed by "functional p53." One can readily determine by routine and well known procedures whether or not a cell is expressing p53 at all. Further, one can readily determine by the same or similar known procedures, if a cell is expressing p53, whether it is functional p53 or a mutated, non-functional version of p53.

Presumptive evidence for the presence of mutant, non-functional p53 can be obtained, for example, by Western blot analysis using monoclonal antibody PAb1801 (Oncogene Science; New York). Such an assay capitalizes on the fact that mutant forms of p53 are more stable than wild-type (functional) p53 and therefore accumulate to much higher protein levels than wild-type p53. Parental cell lines, such as HT1080 cells can be used as a positive control and FM 0097 cells, for example, can be used as a negative control. These materials and methods are described in Anderson et al., *supra*, which is incorporated herein by reference. The presence of wild-type or non-functional mutant forms of p53 can be confirmed by preparing a p53 cDNA clone from the chosen cell line, followed by determining its primary DNA sequence using standard methods well known and available in the art.

The invention also provides methods of enhancing a subject's response to chemotherapy or irradiation by introducing into a subject's tumor cells nucleic acid encoding a polypeptide having adenovirus E1A activity, expressing the E1A active polypeptide in the cells and finally, administering either a chemotherapeutic agent or irradiation.

For enhancing a subject's response to chemotherapy or irradiation, a population of tumor cells can be isolated by a variety of procedures known to those skilled in the art. Isolation is most commonly done surgically by a biopsy. After introduction and expression of E1A in the cells, the new population of E1A-expressing cells are then transferred back into the subject, generally by reinjection or other means well known in the field. Thereafter, tumor regression and/or progression can be monitored by standard methodologies known in the art.

The following examples are intended to illustrate but not limit the invention.

EXAMPLE I

PREPARATION OF STABLE CELL LINES EXPRESSING E1A

Stable E1A-expressing cell lines from HT1080 fibrosarcoma cells and A2058 melanoma cells were constructed using the plasmid p1Aneo as described in Frisch et al., Oncogene, 5:75-83 (1990), which is incorporated herein by reference, Franza et al., Cell, 44:408-418

(1988), which is incorporated herein by reference, and Maruyama et al., Oncogene, 1:361-367 (1987), which is incorporated herein by reference. Cells labeled HT1080neo^r and A2058neo^r resulted from transfection with Bluescript plasmid (Stratagene, La Jolla, CA) containing the simian virus 40 early enhancer-promoted *aph* gene (encoding resistance to G418).

The expression of the E1A gene was documented at the RNA level by Northern blot analysis, also as described in Frisch et al., *supra*. Protein expression was further documented by immunoprecipitation with E1A-specific monoclonal antibodies as follows. Confluent cultures of cell lines (containing 2 x 10^6 cells) were labeled for 5 hours in 35-mm wells with 0.4 mCi (1 Ci = 37 GBq) of [^{35}S] methionine (Tran^{35}S-label, ICN) in methionine-free Dulbecco's modified Eagle's medium containing 5% (vol/vol) dialyzed fetal calf serum. Cells were washed twice in phosphate-buffered saline and scraped into 1.0 ml of RIPA-1 [50 mM Tris-HCl, pH 7.5/0.1% Nonidet P-40/250mM NaCl/aprotinin (10 μg/ml) leupeptin (5 μg/ml)1 mM phenylmethylsulfonyl fluoride/5 mM EDTA/soybean trypsin inhibitor (10 μg/ml)]. After addition of bovine serum albumin to 0.5 mg/ml, lysates were preabsorbed with 100 μl of a 50% (wt/vol) protein A-Sepharose (Pharmacia; Indianapolis, IN) slurry (prepared in RIPA-1 containing bovine serum albumin at 0.5 mg/ml) by mixing at 4°C for 30 minutes and centrifuging for 10 minutes at 14,000 rpm in an Eppendorf microcentrifuge and 0.5 ml samples were then incubated with 1.5 μg of anti-E1A monoclonal antibody M73 (Narlow et al., J. Virol., 3:533-546 (1985)) or control

antibody [anti-fos Ab-1 (Oncogene Sciences, Mineola, NY)] for 2 hours at 0°C. Then 25 μl of 50% protein A-Sepharose slurry was added and the tubes were mixed for 20 minutes at 4°C followed by a 2-minute centrifugation and five 0.5 ml washes with RIPA-1. Pellets were then resuspended in 60 μl of sample buffer and analyzed with SDS/PAGE. E1A protein was detected on the gel as evidenced by Figure 3 which shows the [35S]methionine-labeled proteins from E1A-expressing clones derived from HT1080 cells (lanes 1-5), HeLa cells (lanes 6-8), and A2058 cells (lanes 9-11) immunoprecipitated with anti-E1A antibodies (E) or control antibodies (C). Lanes: 1-5, HT1080, p2AHT2a, p1Aneo3, p1Aneo15, and p1Aneo16, respectively: 6-8, HeLa, Medg18, and Medg28, respectively: 9-11, A2058, 1A58c8-1, and 1A58c11-1, respectively. The bands corresponding to E1A protein species are bracketed.

In addition, construction of E1A viral expression vectors was achieved by subcloning cDNAs encoding the 243 amino acid or 289 amino acid E1A proteins of adenovirus type 5 into the retroviral vectors: LNCX, LNSX. The 243 amino acid form of E1A (12S cDNA) was cut at adenovirus map position 610 with BstX1 and the 5' end was reconstructed with a double-stranded oligonucleotide, thus removing all E1A 5' noncoding sequences. The HindIII end of this oligonucleotide was made blunt ended and the 3' end of E1A was cut with HpaI (map position 1575) to remove the polyA addition site. The resulting E1A sequence was subcloned into the retrovirus vector LNSX (described in Miller and Rosman, Biotechniques, 7:980-990 (1989), which is

incorporated herein by reference, (the retroviral plasmid construct is schematically depicted in Figure 4).

Ten μg of these plasmid DNAs were transfected into the ecotropic packaging cell line gpE86 (maintained in hygromycin/mycophenolic acid selection medium). At 48 hours post transfection, unselected virus stocks were prepared by collecting conditioned media, clearing by centrifugation and freezing. These viral stocks were used to infect the amphotropic packaging cell line gpEam12 (cells available from ATCC) in 35 mm wells by incubation for 12 hours in DME/10% fetal calf serum media containing 4 μg/ml polybrene. At 24 hours post-infection, cells were split at ratios between 1:100-1:300, and infected cells were selected in 500 μg/ml G418 for 3 weeks. Virus-producing cell lines were expanded into 35 mm wells and conditioned media containing virus was prepared for each line. Virus stocks were prepared from producer cell lines containing either 243 amino acid E1A or 289 amino acid E1A, in the vectors LNCX and LNSX (which promote transcription using internal CMV or SV40 early enhancers, respectively).

These stocks were used to infect the human fibrosarcoma line HT1080 by incubating 0.4 ml of virus stock with a 35 mm well of HT1080 cells for 8 hours in medium containing polybrene; infected cells were split at various ratios 24 hours post-infection, and either G418-resistant clones or mixed populations of infected cells were analyzed further. These materials and methods of infecting HT1080 cells with E1A retroviral vectors are also described in Frisch and Francis, J. Cell Biology, 124:619-

626 (1994), which is incorporated herein by reference. The E1A protein expression was confirmed by Western blot analysis as shown in Figure 6 (HT1080 fibrosarcoma (FS) cells expressing E1A (E1A+) compared to HT1080 parental subclone (E1A-)).

Other cell lines were made to express E1A in a similar manner using the above-described retroviral vector infection methods, including Saos-2 osteosarcoma and NCI-H23 non-small-cell lung carcinoma cells.

Abbreviations for the cell lines used hereinafter and in Figures 1 and 6 are as follows: FS: HT1080 fibrosarcoma (subclone H4), ML: A2058 melanoma, OS: Saos-2 osteosarcoma, LC: NCI-H23 non-small cell lung carcinoma, RM: A204 rhabdomyosarcoma, RM2: RD rhabdomyosarcoma, FB: fibroblast.

EXAMPLE II

This example demonstrates the chemotherapeutic drug sensitization effects of E1A with respect to etoposide. Etoposide cytotoxicity dose-response curves of tumor cells and E1A-expressing derivatives are provided.

Twelve-well dishes were inoculated with FS, ML, OS and LC cells and their E1a-expressing counterparts. Subconfluent monolayers were treated with etoposide at 0, 1, 2, 5 and 10 μM (1, 2, 5, 10 and 20 μM for LC cells) for 24 hours. Viable cells were quantitated by MTT assays. These were performed as described by the manufacturer

(Sigma, St. Louis, Missouri). Briefly, cells were incubated at 37°C in medium containing MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) for 1 to 3 hours. The dye crystals were then solubilized with 0.1N HCl in isopropanol. Viability was quantitated by measuring absorbance at 570 nm.

As shown in Figure 1, E1A caused a remarkable degree of sensitization to killing by etoposide, significantly more E1A-expressing tumor cells were killed by etoposide than the parental cells lacking E1A. Because at least FS, OS, and LC cells do not express functional p53, Anderson et al., *supra*, Subler and Martin, *supra*, and Takahashi et al., *supra*, respectively, the observed effect of enhanced sensitization is independent of the p53 protein.

EXAMPLE III

This example further demonstrates the effect of E1A on the sensitivity of A2058 human melanoma cells and the E1A-expressing cell's response to etoposide. Here, the degree of sensitization to killing by this drug is determined by gel electrophoresis of DNA extractions.

Parental A2058 human melanoma cells lacking E1A (E1A-) and the E1A-expressing derivative cell line 1A58c8-1 (E1A+) both were plated in 60mm dishes and grown to confluence. Etoposide (Sigma, St. Louis, Missouri) was added to a final concentration of 25 μM and incubation was continued for another 24 hours. Low-molecular weight DNA

was extracted with 0.5% Triton X-100, 10 mM EDTA, and 10 mM Tris, pH 7.4 (0.6 ml), phenol-chloroform extracted three times, ethanol precipitated and analyzed on a 1.5% agarose gel in TBE buffer.

As demonstrated in Figure 2, there is no DNA degradation, indicative of cell death, in cells lacking E1A, even where etoposide is present (E1A-, etoposide-; E1A-, etoposide+). And while there is evidence of some DNA degradation and killing with E1A alone (E1A+, etoposide-), with the methods of this invention there is a striking increase in DNA degradation and hence the response of human melanoma cells to etoposide in the presence of E1A (E1A+; etoposide+). Therefore, E1A converts partially or totally drug-resistant tumors such as A2058 melanoma into drug-sensitive tumors.

Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific examples detailed are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

I claim:

1. A method of treating a human tumor cell, comprising the steps of:

a. introducing into said tumor cell nucleic acid encoding a polypeptide having E1A activity;

b. expressing said polypeptide in said tumor cell; and

c. contacting said tumor cell with a chemotherapeutic agent.

2. The method of claim 1, wherein the nucleic acid encodes adenovirus E1A polypeptide.

3. The method of claim 1, wherein the introducing is effected in vitro.

4. The method of claim 1, wherein the introducing is effected in vivo.

5. The method of claim 1, wherein the nucleic acid is introduced into the tumor cell by contacting the cell with a suitable retroviral vector containing the nucleic acid sequence.

6. A method of claim 1, wherein the chemotherapeutic agent is selected from the group consisting of etoposide, adriamycin, amsacrine, actinomycin D, VP16, camptothecin, colchicine, taxol, cisplatinum, vincristine, vinblastine, and methotrexate.

7. A method of treating a human tumor cell, comprising the steps of:

a. introducing into said tumor cell nucleic acid encoding a polypeptide having E1A activity;

b. expressing said polypeptide in said tumor cell; and

c. irradiating said tumor cell.

8. The method of claim 7, wherein the nucleic acid encodes adenovirus E1A polypeptide.

9. The method of claim 7, wherein the introducing is effected in vitro.

10. The method of claim 7, wherein the introducing is effected in vivo.

11. The method of claim 7, wherein the nucleic acid is introduced into the tumor cell by contacting the cell with a suitable retroviral vector containing the nucleic acid sequence.

12. A method of enhancing a response to chemotherapy in a subject, comprising the steps of:

a. introducing into said subject's tumor cells nucleic acid encoding a polypeptide having E1A activity;

b. expressing said polypeptide in said tumor cells; and

c. administering a chemotherapeutic agent to said subject.

13. The method of claim 12, wherein the introducing step (a) is by direct injection into said subject.

14. The method of claim 12, wherein the introducing step (a) is on a population of tumor cells which have been isolated from a subject which are subsequently transferred back into said subject.

15. The method of claim 12, wherein the nucleic acid encodes adenovirus E1A polypeptide.

16. A method of enhancing a response to irradiation in a subject, comprising the steps of:

a. introducing into said subject's tumor cells nucleic acid encoding a polypeptide having E1A activity;

b. expressing said polypeptide in said tumor cells; and

c. administering irradiation to said subject.

17. The method of claim 16, wherein the introducing step (a) is by direct injection into said subject.

18. The method of claim 16, wherein the introducing step (a) is on a population of tumor cells which have been isolated from a subject which are subsequently transferred back into said subject.

19. The method of claim 16, wherein the nucleic acid encodes adenovirus E1A polypeptide.

20. A method of treating a cancer which is susceptible to treatment, comprising the steps of:

a. introducing into a subject's tumor cells nucleic acid encoding a polypeptide having adenovirus E1A activity;

b. expressing said polypeptide in said tumor cells; and

c. contacting said tumor cells with a chemotherapeutic agent.

21. A method of treating a cancer which is susceptible to treatment, comprising the steps of:

a. introducing into a subject's tumor cells nucleic acid encoding a polypeptide having adenovirus E1A activity;

b. expressing said polypeptide in said tumor cells; and

c. irradiating said tumor cells.

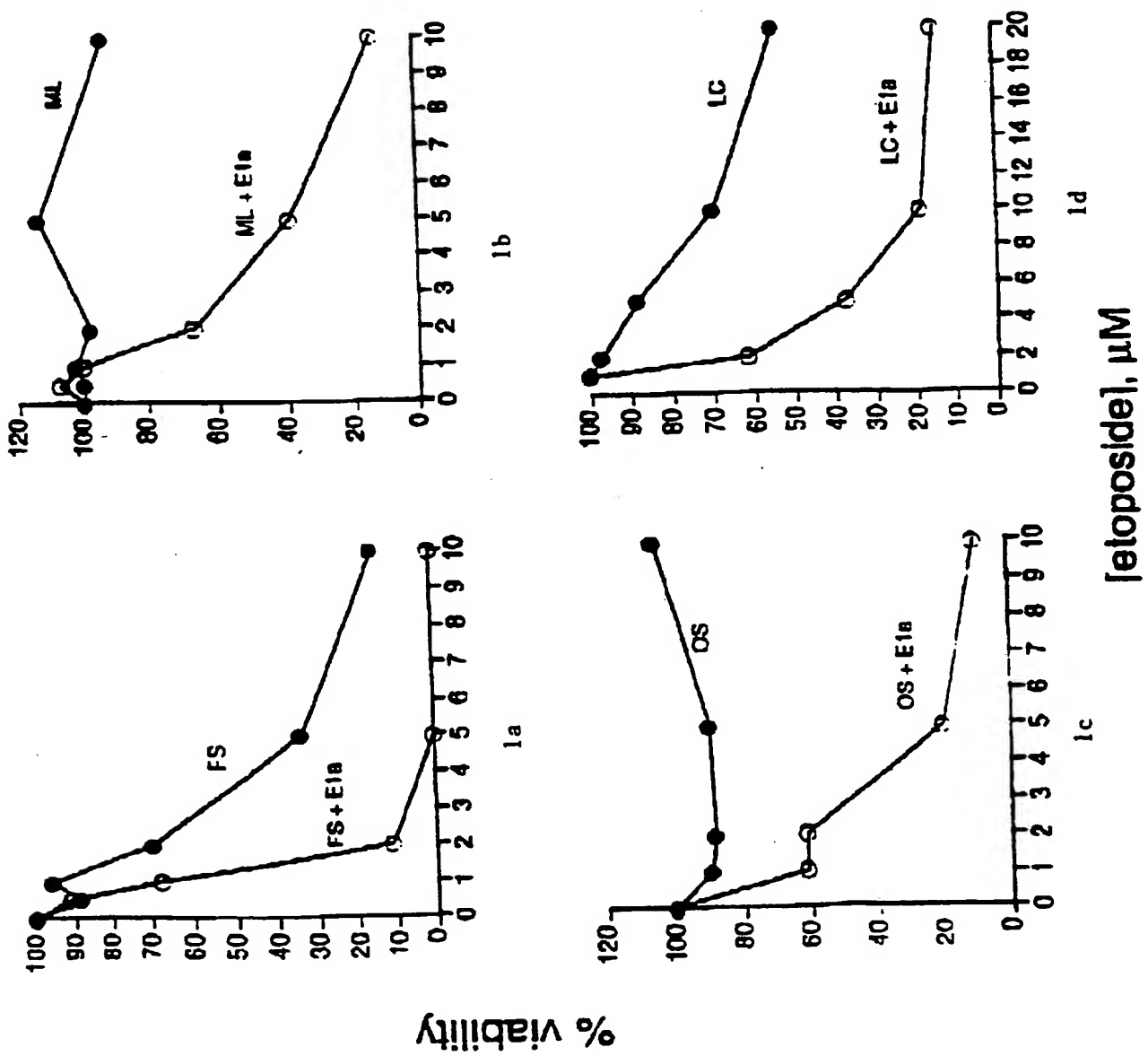

% viability
[etoposide], µM
FS
FS+E1a
1a
ML
ML+E1a
1b
OS
OS+E1a
1c
LC
LC+E1a
1d

FIGURE 1

Figure 2

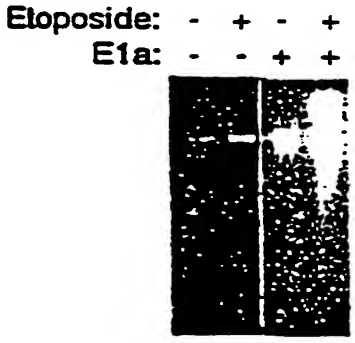
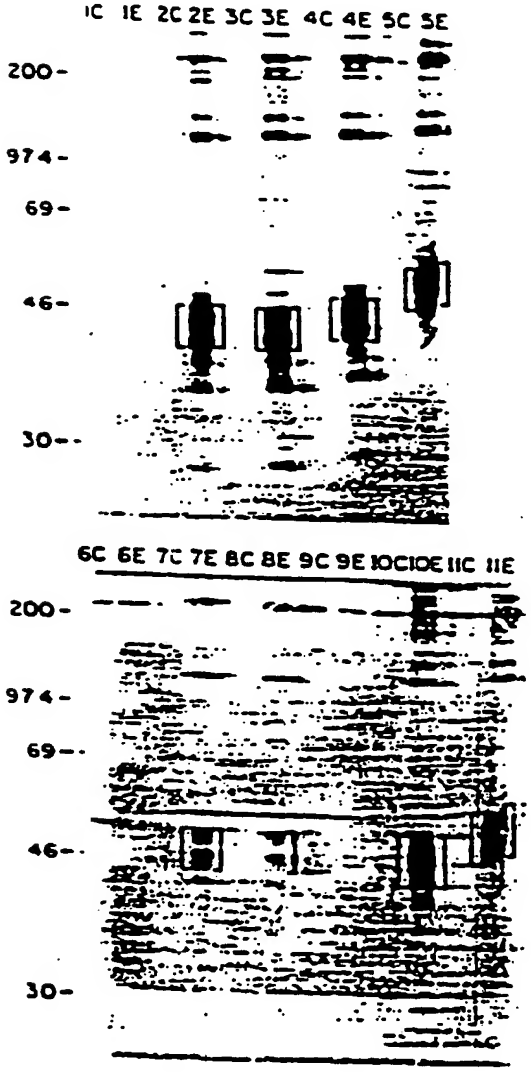


Figure 3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200-
97.4-
69-
46-
30-
6C 6E 7C 7E 8C 8E 9C 9E 10C 10E 11C 11E
200-
97.4-
69-
46-
30-

Figure 4

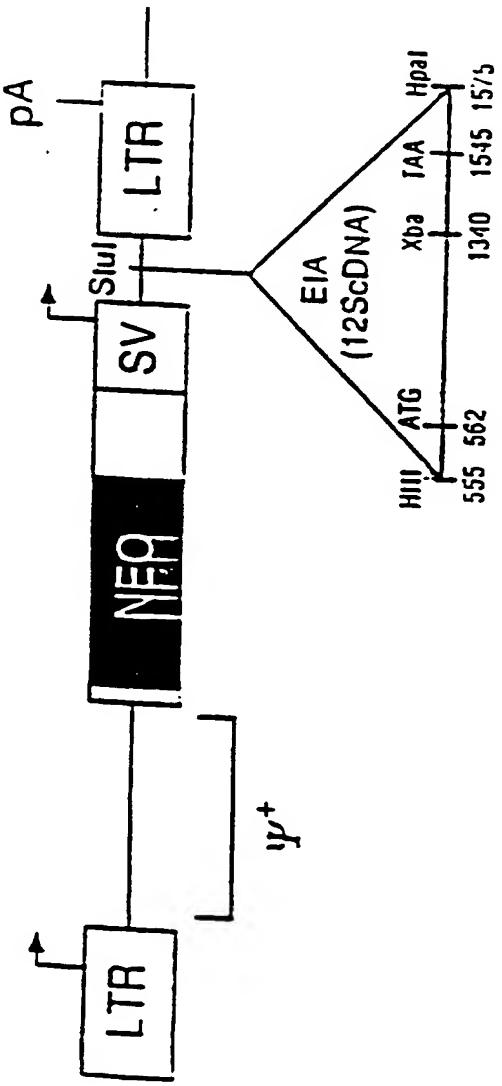


FIGURE 5

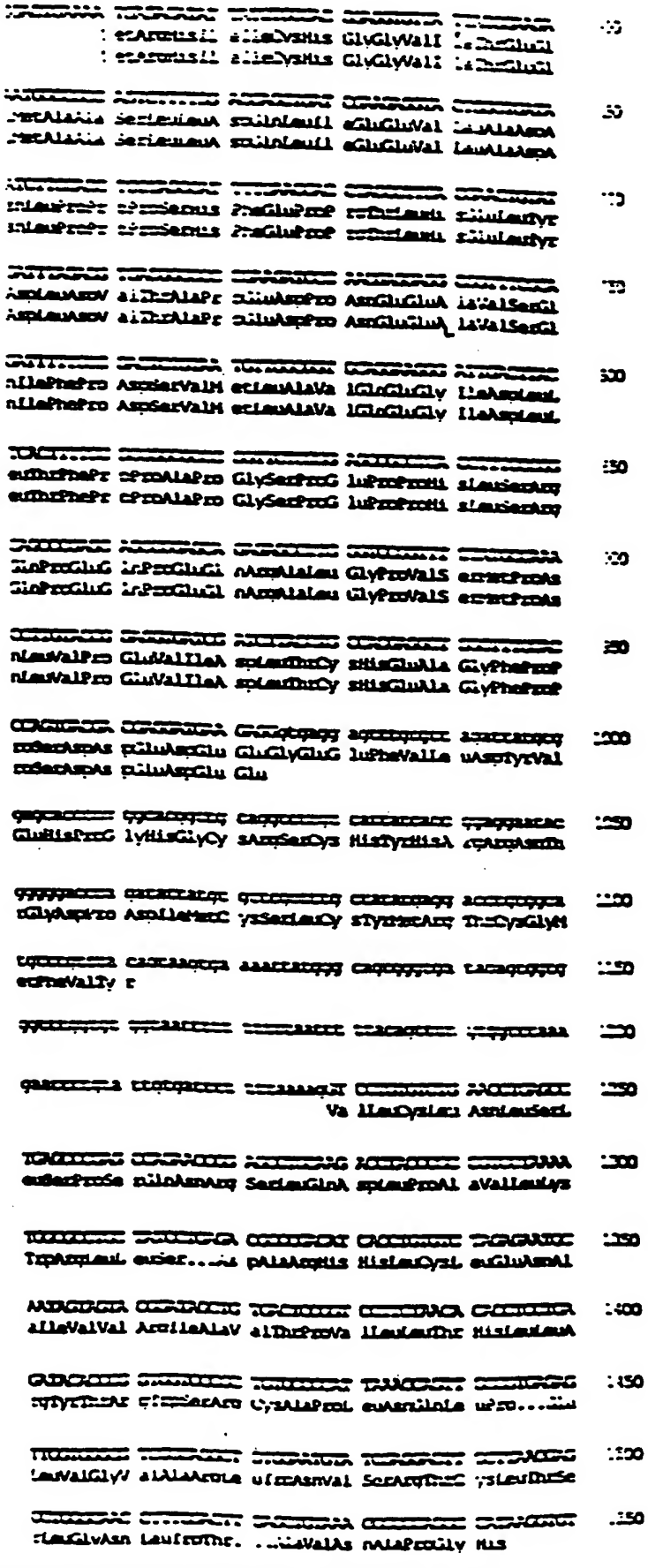
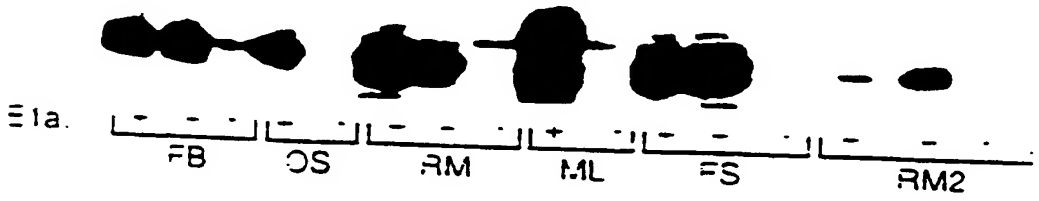


Figure 6



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/11342

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :424/93.21; 435/172.3, 240.2, 320.1; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.21; 435/172.3, 240.2, 320.1; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE
Search terms: Frisch, E1A, adenovirus, adenoviral, chemotherapy, radiation, radiotherapy, apoptosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences USA, Volume 88, issued October 1991, Frisch, "Antioncogenic effect of adenovirus E1A in human tumor cells", pages 9077-9081, see entire document.	1-21
Y	Proceedings of the National Academy of Sciences USA, Volume 89, issued August 1992, Rao et al., "The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins", pages 7742-7746, see entire document.	1-21
Y	Cell, Volume 74, issued 1993, Lowe et al., "p53-dependent apoptosis modulates the cytotoxicity of anticancer agents", pages 957-967, see entire document.	1-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 OCTOBER 1995	Date of mailing of the international search report 28 NOV 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Deborah Reese for MICHAEL J. NEWELL Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/11342

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	International Journal of Cancer, Volume 60, issued 1995, Sanchez-Prieto et al., "Modulation of chemical chemoresistance in keratinocytes by activation of different oncogenes", pages 235-243, see entire document.	1-21
Y	International Archives of Allergy and Immunology, Volume 105, issued 1994, Gruber et al., "Apoptosis and therapy of malignant diseases of the hematopoietic system", pages 368-373, see entire document.	1-21
Y	Leukemia, Volume 8, Supplement 1, issued April 1994, Sincovics et al., "Apoptosis by genetic engineering", pages S98-S102, see entire document.	1-21
Y, P	Seminars in Cancer Biology, Volume 6, issued 1995, Canman et al., "Induction of apoptosis by tumor suppressor genes and oncogenes", pages 17-25, see entire document.	1-21
Y	Current Opinion in Oncology, Volume 6, issued 1994, Martin et al., "Apoptosis as a goal of cancer therapy", pages 616-621, see entire document.	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/11342

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01N 43/04; A61K 48/00; C12N 5/10, 7/00, 15/00, 15/09, 15/33, 15/34